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The morphology, phylogeny, and distribution of the pine-associated sequestrate fungus *Lactarius cinnabarinus* comb. nov. (= *Zelleromyces cinnabarinus*) in the United States, Argentina and Brazil

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Abstract. The sequestrate fungus *Zelleromyces cinnabarinus* was originally found beneath pines and was described from Louisiana, USA. Here we re-evaluate the morphology, taxonomy, and distribution of this species based on new specimens across the native range in the southeastern United States (USA), as well as introduced populations in Argentina and Brazil. Internal Transcribed Spacer (ITS) sequences of fungarium specimens indicate that this species is widespread in the southeastern USA with native pines and has been introduced to South America in cultivated pine plantations. An environmental sequence from ectomycorrhizal *Pinus* roots also confirms the symbiotic association with pines. Our data also indicate that *Lactarius taedae*, a sequestrate fungus recently described from Brazil, is a later synonym of *Zelleromyces cinnabarinus*. Lastly, our phylogenetic analysis of ITS sequences suggests that *Zelleromyces cinnabarinus* is nested within the genus *Lactarius*, so we recombine this sequestrate species as *Lactarius cinnabarinus* comb. nov.

Key words: angiocarpic basidiomes, hypogeous fungi, new combination, phylogenetic comparison

Introduction

Gasteroid milkcaps are hypogeous and angiocarpic fungi that produce abundant latex when cut (Singer & Smith 1961). These fungi have historically been described in two genera, *Arcangeliella*, with subagaricoid basidiomata with a stipe and columella, and *Zelleromyces* with gasteroid basidiomata that lack a stipe and columella (Singer & Smith 1960; Sundberg & Trappe 1975; Eberhardt & Verbeken 2004; Vidal et al. 2004; Lebel et al. 2021). However, recent research has questioned this classification (Vidal 2004; Eberhardt & Verbeken 2004; Lebel et al. 2021). In recent years, phylogenetic analyses have shown that some gasteroid milkcaps are more closely related to three genera within the *Russulaceae* family: *Lactarius*, *Russula*, and *Lactifluus* (Calonge & Martin 2000; Nuytinck et al. 2003; Eberhardt & Verbeken 2004; Lebel et al. 2021). Furthermore, the gasteroid hypogeous milkcap species included in *Arcangeliella* and *Zelleromyces* do not form a monophyletic clade, but are instead nested among the agaricoid species of *Russulaceae* (Nuytinck et al. 2003; Eberhardt & Verbeken 2004).

Accordingly, several species originally named in *Acangeliella* have been transferred to *Lactarius*, such as the type species of this genus, *Lactarius borzianus* (Nuytinck et al. 2003). Similarly, several species originally named in *Zelleromyces* were transferred to *Lactarius* and other gasteroid milkcaps have been described as new species in *Lactarius*. The list of species that follow this pattern include: *Lactarius angiocarpus* (Eberhardt & Verbeken 2004), *L. gardneri*, *L. giennensis* (Pierotti 2015), *L. josserandii*, *Lactarius rubriviridis* (Desjardin 2003), *L. soehneri* (Vidal et al. 2019), and *L. stephensii* (Nuytinck et al. 2003). In Australasia there are a few species of sequestrate fungi that belong to the related latex-producing genus *Lactifluus*, although sequestrate

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taxa are less common in this genus (Lebel et al. 2021). There is also at least one case where a sequestrate species was originally named in *Zelleromyces*, but belongs to the genus *Russula*. That species is now considered under the name *R. meridionalis* (Vidal et al. 2019).

As part of a project studying the fungi associated with planted pine forests in northeastern Argentina, a freshly collected specimen was morphologically identified as *Zelleromyces cinnabarinus*. Preliminary morphological observations and ITS sequence data suggested that the specimen from Argentina was also quite similar to another species that was recently described from Brazil: *L. taedae* (Silva-Filho et al. 2018). To clarify the identity and morphology of *Z. cinnabarinus*, we examined the type specimen of *Z. cinnabarinus* and also studied recent collections of this taxon from across the Southeastern USA, including specimens from near the type locality in Louisiana. Here, we recombine this sequestrate fungus as *Lactarius cinnabarinus* comb. nov. and provide an update on the morphology, taxonomy, and phylogeny of *L. cinnabarinus* from its native range in the Southeastern USA and with introduced pines in Argentina and Brazil.

Material and methods

Collections and morphological analyses

The TYPE specimen of *Zelleromyces cinnabarinus* was obtained on loan from the University of Michigan (MICH) and another more recent collection identified as *Z. cinnabarinus* from Louisiana was obtained on loan from the Field Museum of Natural History (F). Additional specimens from recent collections identified as *Z. cinnabarinus* from Florida, Louisiana, and North Carolina are housed at the Fungarium of the Florida Museum of Natural History (FLAS) at the University of Florida. A new specimen from planted *Pinus taeda* in Corrientes province, Argentina, was identified as *Z. cinnabarinus* and deposited at the Instituto de Botánica del Nordeste, Argentina (CTES). New specimens were photographed when fresh and macroscopically described according to Vidal et al. (2019). The color codes follow Kornerup & Wanscher (1978). Sulfo-phospho-vanillin and KOH (5%) were used to observe color changes in the basidiomata. Microscopic characters were examined using a DM500 Leica Microsystems (Deerfield, IL, USA) light microscope (LM) and a 5800 LV JEOL (Peabody, MA, USA) scanning electron microscope (SEM) operating at 20 KV. All LM images were taken with a Leica ICC50W camera from material mounted in 5% KOH and stained with phloxine (1%) or treated with Melzer's reagent. SEM images were obtained from fungarium samples, rehydrated in Triton aqueous solution, and dehydrated in an ethanol series, critical-point dried, and then mounted on double-sided tape and coated with gold-palladium. The measurements were made directly using LM or through photographs using ImageJ software (Schneider et al. 2012). We measured the length and width or the diameter of microstructures (basidiospores, basidia, cystidia, peridiopellis, terminal elements). Spore ornaments were measured using SEM. For basidiospore measurements, ornamentation was excluded and the following notations were used: L(W) = length (width) average from a single basidioma; Q = quotient of length and width; Q_x = mean of Q values; n = number of basidiospores measured, N = number of basidiomata. New data was analyzed and compared with the keys and descriptions from Singer & Smith (1961), Sundberg & Trappe (1975), Silva-Filho et al. (2018) and Vidal et al. (2019). Herbarium abbreviations follow Index Herbariorum (Thiers 2023) and the authors of species are according to Index Fungorum – Authors of Fungal Names (2023).

DNA extraction, amplification, and sequencing

Genomic DNA was isolated from dried basidiomata tissue following standard protocols of the Canadian Centre for DNA barcoding (CCDB) for fungi (Ivanova et al. 2006, 2016) or using a rapid alkaline extraction procedure (Rennick et al. 2023). The nuclear ribosomal internal transcribed spacer (nrITS) region of the DNA was amplified using primers ITS1–F and ITS4–B (Gardes & Bruns 1993), which was suggested as the universal DNA barcode marker for fungi (Ivanova et al. 2008; Schoch et al. 2012). PCR products were purified and sequenced using the Sanger sequencing method by the Canadian Center of DNA Barcoding (CCDB) or at Eurofins Genomics (Louisville, KY, USA). All DNA sequences were deposited into the GenBank database (Table 1).

Phylogenetic analyses

New sequences from *Z. cinnabarinus* specimens were assembled and manually edited using Geneious v. 6.1.8 (Kearse et al. 2012). The nrITS dataset was built with sequences of *Lactarius* subg. *Russularia* treated by Silva-Filho et al. (2018) and Vidal et al. (2019), as well as sequences generated from this study and closely related sequences that were obtained via BLAST searches (Table 1). *Lactarius torminosus*, *L. scrobiculatus* and *L. purpureus* from *Lactarius* subg. *Lactarius* were used as the outgroup (Wisitrassameewong et al. 2016). The nrITS sequences were aligned with MUSCLE 3.8.425 (Edgar 2004) using default settings in Geneious 2020.2.4 (Auckland, New Zealand) and edited manually. Ambiguously aligned regions were removed from all alignments with Gblocks (Talavera & Castresana 2007) using the least stringent settings. The best fit model of nucleotide evolution was selected using AIC (Akaike Information Criterion) as implemented in jModelTest2 v.1.6 (Guindon & Gascuel 2003; Darriba et al. 2012). Maximum Likelihood (ML) phylogenetic analysis was performed with RAxML-NG (1.2.0) (Stamatakis 2014), and support for branches was evaluated with 1,000 bootstrap pseudoreplicates. jModelTest2 v.1.6 and RAxML-NG (1.2.0) were used on the CIPRES Science Gateway (Miller et al. 2010; http://www.phylo.org/). A node was considered to be strongly supported if it showed a bootstrap support ≥ 70%.

Results

The nrITS dataset included 50 sequences belonging to species of *Lactarius* subg. *Russularia* and 3 outgroup

Table 1. Specimens, voucher collection, origin, GenBank accession numbers of ITS sequences and references used in the molecular analyses. Sequences added in this work are highlighted in bold.

Species	Voucher collection	Origin	ITS accession numbers	References
Alnus ECM root tip	Isolate S761	China	HE979371	Põlme et al. (2013)
Arcangeliella sp.	BH2148F	Tasmania	JF960610	Horton (2011)
Lactarius atrii	AVKDKVP-09-066	Thailand	KT165241	Wisitrassameewong et al. (2016)
Lactarius badiopallescens	LM4629F	Mexico	FJ348706	Montoya et al (2010)
Lactarius borzianus	16944	USA	JF908775	Osmundson et al. (2013)
Lactarius camphoratus	UE04.09.2004-5	Sweden	DQ422009	Eberhardt (unpubl.)
Lactarius chrysorrheus	UE04.10.2002-8	Italy	KF133261	Verbeken et al. (2014)
Lactarius cyathuliformis	UE04.09.2004-2	Sweden	KF133266	Verbeken (2014)
Lactarius decipiens	AV2000-137	Thailand	KF432973	Wisitrassameewong et al. (2014)
Lactarius eucalypti	MEL2238273	Australia	EU019923	Lebel & Tonkin (2007)
Lactarius flavigalactus	AVKDKVP-09-021	India	KT165264	Wisitrassameewong et al. (2016)
Lactarius fulvissimus	JKLAC10082001	Germany	KF432970	Wisitrassameewong et al. (2016)
Lactarius helvus	UE08.09.2004-1	Sweden	KF133263	Verbeken et al. (2014)
Lactarius hepaticus	M17	Netherlands	EU924787	Warmink et al. (2009)
Lactarius imperceptus	JMP0044	USA	EU819485	Palmer et al. (2008)
Lactarius inconspicuus	KW100	Thailand	KF433001	Hampe & Verbeken (2015)
Lactarius josserandii	JMV800621	Spain	MK105613	Vidal et al. (2019)
Lactarius josserandii	MA-Fungi 38311	Spain	AF231913	Calonge & Martín (2000)
Lactarius joserandii	MA-Fungi 37498	Spain	AF231912	Calonge & Martin (2000)
Lactarius kesiyae	KW032	Vietnam	KR025619	Hampe & Verbeken (2015)
Lactarius kesiyae	CUH AM810	India	OR592288	Thapa et al. (unpubl.)
Lactarius kesiyae	HKAS122579	China	ON794380	Wang et al. (2022)
Lactarius kesiyae	SFC20150701-62	South Korea	MG551729	Lee et al. (2019)
Lactarius lachungensis	AVKDKVP-09-019	India	KT165284	Wisitrassameewong et al. (2016)
Lactarius lanceolatus	I23	USA	KR090915	Barge et al. (2016)
Lactarius luculentus	I30	USA	KR090922	Barge et al. (2016)
Lactarius purpureus	FH12-008	Thailand	KF432966	Wisitrassameewong et al. (2014)
Lactarius quietus	UE16.09.2004	Sweden	KF133264	Verbeken et al. (2014)
Lactarius rubrocinctus	EDC12-210	Thailand	KF432977	Wisitrassameewong et al. (2014)
Lactarius scrobiculatus	JN01-058	Slovakia	KF432968	Wisitrassameewong et al. (2014)
Lactarius sikkimensis	AVKDKVP-09-024	India	KT165252	Wisitrassameewong et al. (2016)
Lactarius silviae	74231	USA	EU644700	Molina et al. (2001)
Lactarius sphagneti	PL2805	UK	KF133268	Verbeken et al. (2014)
Lactarius subdulcis	JV2006-024	Belgium	KF133279	Verbeken et al. (2014)
Lactarius sublaccarioides	KW011	Thailand	KF432999	Wisitrassameewong et al. (2014)
Lactarius taedae	SMDB 18030	Brasil	NR163296	Silva-Filho et al. (2019)
Lactarius taedae	M.A. Sulzbacher510	Brasil	MG873443	Silva-Filho et al. (2019)
Lactarius torminosus	JN11-086	Greece	KR025613	Wisitrassameewong et al. (2015)
Pinaceae root tip	yi5A4sp2	Taiwan	KC679829	Chen (2013)
Pinaceae root tip	yi3A4sp2	Taiwan	KC679828	Chen (2013)
Pinus taeda ECM	125.1ue_ITS	USA	KY651145	Ulyshen et al. (2017)
Zelleromyces claridgei	MEL 2236358	Australia	NR173480	Lebel et al. (2021)
Zelleromyces corkii	MEL 2236387	Australia	NR174669	Lebel et al. (2021)
Zelleromyces sp. 1	BH3528R	Tasmania	JF960852	Horton (2011)
Zelleromyces sp. 2	BH2174F	Tasmania	JF960853	Horton (2011)
Zelleromyces sp. 3	BH1714P	Tasmania	JF960854	Horton (2011)
Zelleromyces cinnabarinus	VSC F 0000101	USA	ON080954	Cantonwine et al. (unpubl.)
Zelleromyces cinnabarinus	FLAS-F-60604	USA	MH016796	Kaminsky et al. (unpubl.)
Zelleromyces cinnabarinus	AS 17	Argentina	**OQ658761**	**This work**
Zelleromyces cinnabarinus	DPL11101	USA	**PP238187**	**This work**
Zelleromyces cinnabarinus	FLAS:F-72161	USA	**PP337373**	**This work**
Zelleromyces cinnabarinus	DPL11101	USA	**PP238187**	**This work**
Zelleromyces cinnabarinus	FLAS-F-63103	USA	**PP235416**	**This work**

sequences of *L.* subg. *Lactarius*, resulting in an alignment with 595 characters, of which 145 are parsimony-informative. The best substitution model was estimated as GTR+I+G.

Phylogenetic inference from nrITS (Fig. 1) shows the genus *Zelleromyces* is polyphyletic, with several sequestrate species interspersed in *Lactarius* subg. *Russularia* with good support. Our specimens of *Z. cinnabarinus* fell into a well-supported clade (BS = 100) (Fig. 1). This clade includes morphologically similar fungarium specimens from Louisiana (near the TYPE locality) and other sites across the Southeastern USA (Florida and North Carolina). Two sequences from GenBank were also nested in the *Z. cinnabarinus* clade: an environmental sequence

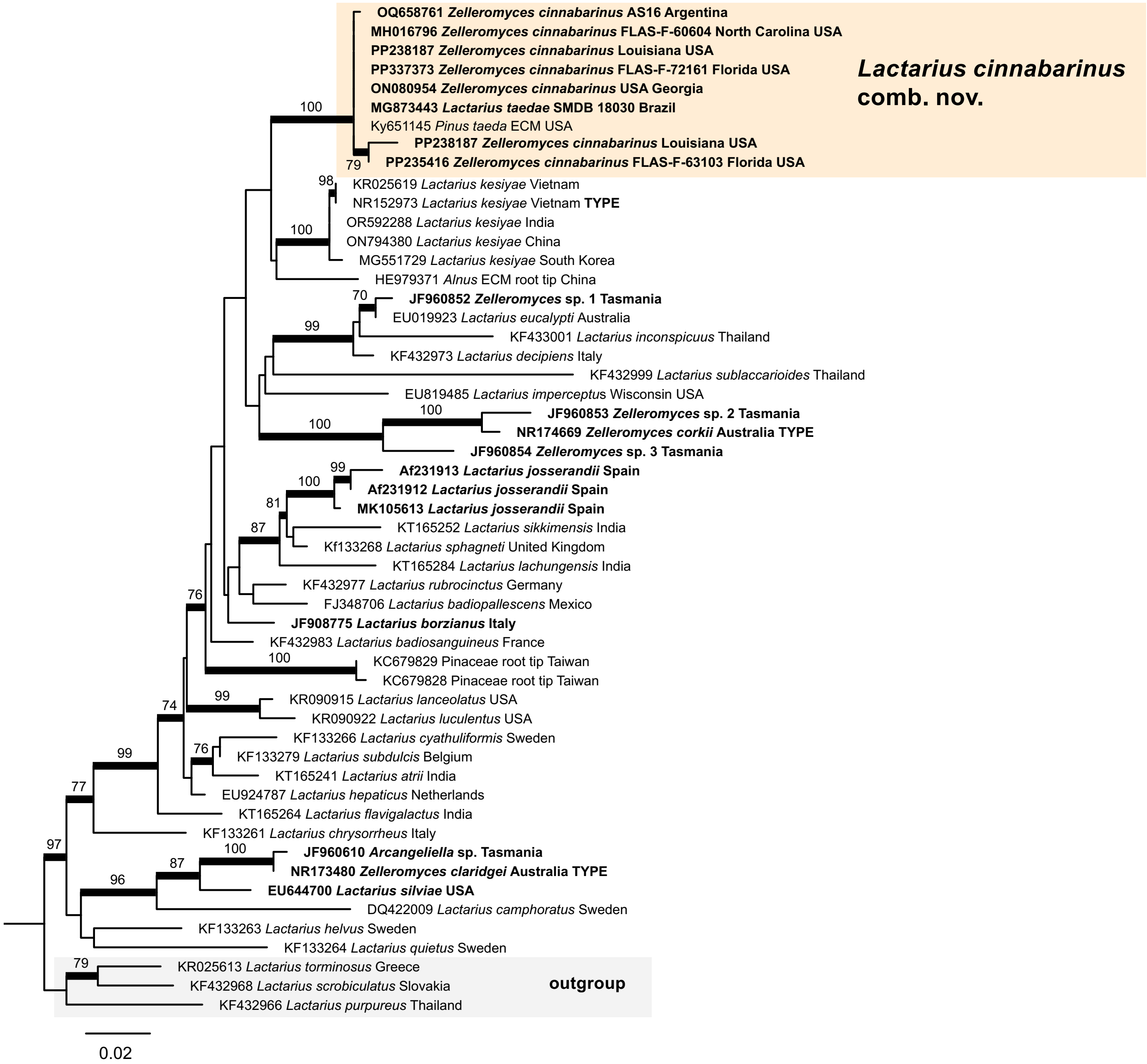

Figure 1. Maximum likelihood (ML) tree from nrITS dataset. Support values at the nodes are shown for bootstrap (BS) ≥70. Thicker lines in black represent supported nodes. The clade with taxa of interest is highlighted. Specimens of sequestrate species are indicated in bold text and type specimens are indicated with "TYPE".

from an ectomycorrhizal root tip of *Pinus taeda* growing in Mississippi (KY651145; Ulyshen et al. 2017) and a fungarium specimen from Valdosta, Georgia (specimen VSC-F-0000101 identified by Dr. Emily Cantonwine, GenBank ON080954). Additional South American sequences in this clade included our new specimen from Argentina (AS 17, CTES) from a pine forest in Corrientes and specimens of the newly described *Lactarius taedae* from southern Brazil.

In addition to *Z. cinnabarinus*, the ITS sequences from two additional species of *Zelleromyces* (*Z. claridgei* and *Z. corkii*) are well supported within the genus *Lactarius* (Fig. 1).

Taxonomy

Lactarius cinnabarinus (Singer & A.H. Sm.) Somrau & M.E. Sm., comb. nov. (Figs 2, 3)

MycoBank MB 852106

Basionym: *Zelleromyces cinnabarinus* Singer & A.H. Sm., Mem. Torrey Bot. Club 21(3): 19. 1961.

Type: United States, Louisiana, East Feliciana Parish, Jackson, under young pines, 26 December, 1931. A.H. Smith & C.A. Brown (holotype, MICH 12420!)

Synonym: *Lactarius taedae* Silva-Filho, Sulzbacher & Wartchow, Phytotaxa 379(3): 236. 2018.

Type: Brazil, Rio Grande do Sul, Santa Maria, Campus da Universidade Federal de Santa Maria, associated with *Pinus taeda*, 17 May 2017, M.A. Sulzbacher 510 (SMDB 18030; UFRN-Fungos 2950!) *ibid*, 17 May 2017, M.A. Sulzbacher 511 (SMDB 18031; UFRN-Fungos 2951!).

Description. Basidiomata gasteroid, angiocarpic, 15.0–50.0 mm wide, tuberiform, subglobose to oval. Peridium persistent, smooth, shiny when wet, mature specimens reddish-orange to reddish-brown (7B7–8D8) with lighter punctuations on top, young specimens pastel red to orange-brown (7A5–7C8). Gleba labyrinthoid, loculated, irregular, columns 1.5–2.0 × 1.0–2.0 mm, flattening towards the edges, empty, grayish-orange to reddish-white when young

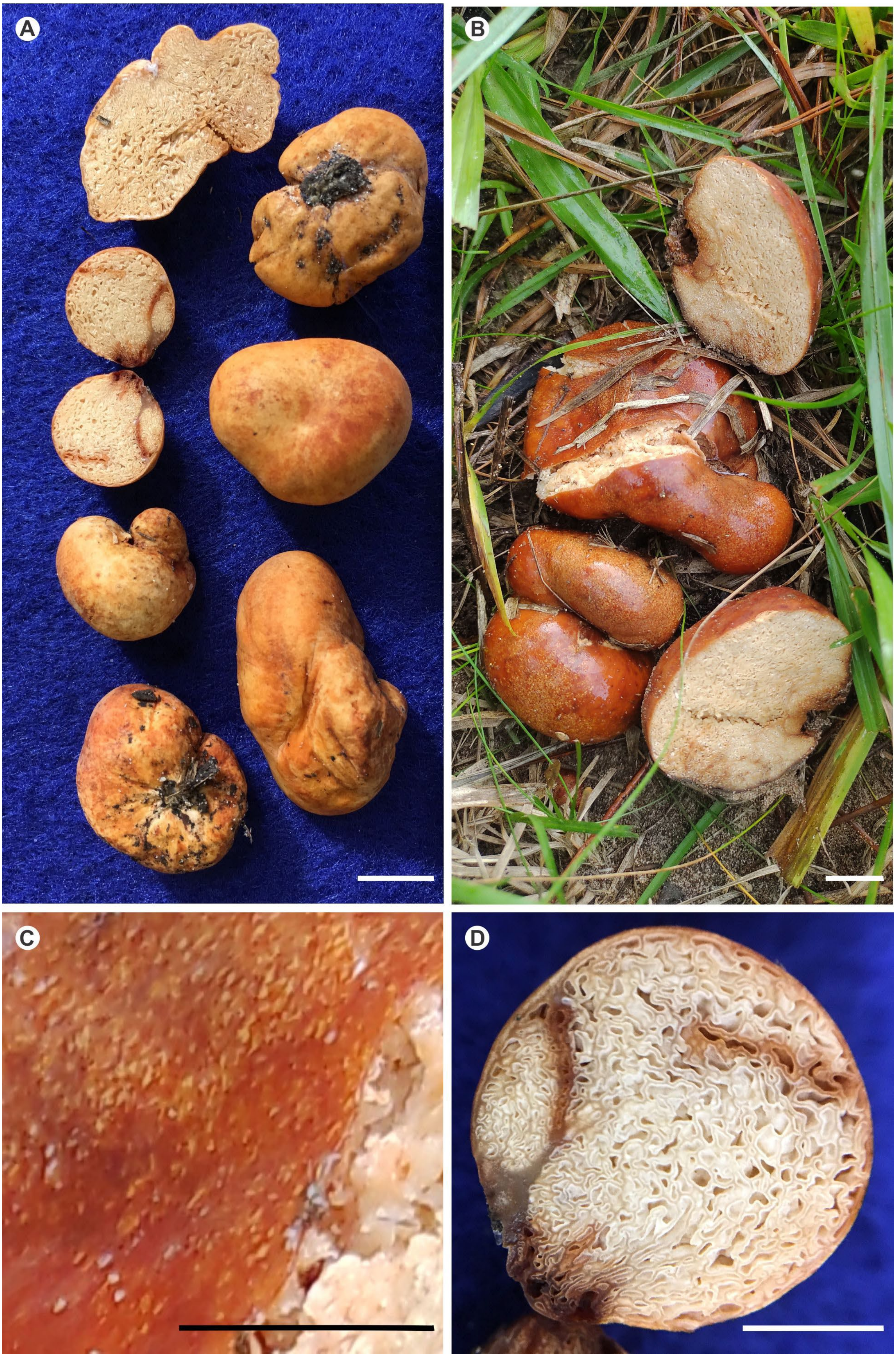

Figure 2. *Lactarius cinnabarinus* basidiomata. A, B – general aspect of the basidiomata; C – detail of the peridium, where color punctuations can be appreciated; D – detail of the gleba. Scales: A, B = 10 mm; C, D = 5 mm.

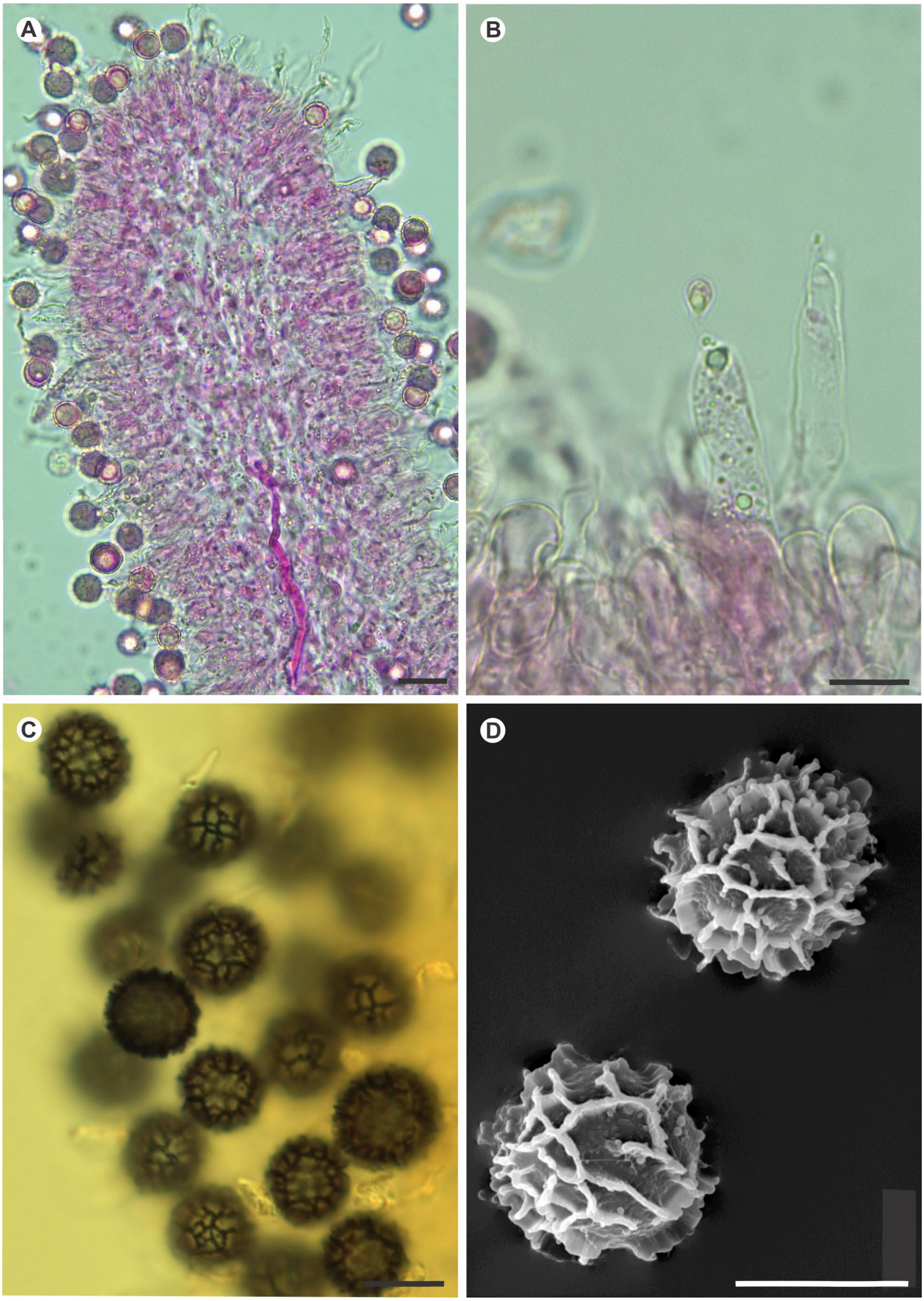

Figure 3. *Lactarius cinnabarinus* micrographs of the internal structures and SEM micrographs of the basidiospores. A – transversal cut of the basidiomata; B – detail of a 1–spored basidium; C – spores from the type material (MICH 12420); D – spores under SEM. Scale = 10 μm.

(6B3–7A2) and brown to light brown (7D7) when mature or bruised. Latex scant to profuse, white, unchanging, more abundant in the pileal context. Columella rudimentary to absent. Mild taste with farinaceous texture, woody aroma.

Basidiospores 10.3–15.7 × 10.1–14.3 µm (excluding ornamentation), L = 12.1 ± 1.0 µm, W = 11.4 ± 0.8 µm, Q = 1–1.14, Q_x = 1.05, n = 30; globose to subglobose; reticulated ornamentation, with a strong amyloid reaction. Reticulum 1.2 to 2.5 µm high, formed by broad ridges fused typically in long chains, with ridged to warted margins. Basidia 47.8–76.9 × 6.0–14.6 µm, clavate to cylindrical-clavate, arising from the subhymenium, thin–walled, collapsing once the spores are released; predominantly 2–spored, occasionally 1–, 3–, and 4–spored (with no appreciable differences in the size observed between basidia with different numbers of basidiospores), hyaline, sometimes with refractory, inamyloid, yellowish content of variable sizes, from small punctuations forming clumps to large spheres. Cystidia not observed. Peridiopellis a cutis of repent, tightly woven hyphae of 2.0–5.0 µm diam., hyaline or sometimes with yellowish refractory content; occasionally forming short to clavate, semi-erect to ascending terminal elements. Terminal elements pointed, 19.0–36.0 × 3.5–5.0 µm, thin-walled, hyaline. Lactiferous hyphae abundant in the gleba and strongly contrasting when viewed in Melzer's reagent. Sphaerocytes and clamp-connections absent in all examined tissues.

Habitat. Hypogeous to subhypogeous in pine forests, mixed oak-pine forests, and pine plantations.

Specimens examined. ARGENTINA. Corrientes, Ituzaingó, in the surroundings of the Yerbalito Ecological Reserve in *Pinus taeda* plantations, 22 May 2022. A. Somrau AS17 (CTES). USA. Florida, Alachua Co., Gainesville, campus of the University of Florida, Natural Area teaching lab in mixed pine-oak woods, 30 December 2018. S. Prentice, N. Kraisitudomsook, J. Martin (FLAS-F-63103). Franklin Co., 4 December 2023, Dr. Julian G. Bruce St. George Island State Park, in a coastal *P. elliottii* forest, B. Lemmond, BL644 (FLAS-F-72161). Louisiana, East Feliciana Parish, Jackson, under young pines, 26 December, 1931. A.H. Smith & C.A. Brown (holotype, MICH 12420); Beauregard Parish, Longville, in a trailer park, 10 December 2013. D.P. Lewis 11101 (C0304730F). North Carolina, Durham Co., Durham, off Garrett Road in mixed pine forest, 15 November 2009. M.E. Smith, F. Smith, O. Smith, MES-336 (FLAS-F-60604).

Distribution. Eastern North America, confirmed across the southeastern states and likely present further north (Sundberg & Trappe 1975), introduced in Argentina and Brazil (Silva-Filho et al. 2018, as *L. taedae*).

Discussion

Lactarius cinnabarinus is a sequestrate ectomycorrhizal fungus that was originally described as *Zelleromyces cinnabarinus* from Louisiana USA, where it was collected under young pines (Singer & Smith 1961). This species was originally reported based only on the type specimen from Louisiana until Sundberg & Trappe (1975) documented additional, morphologically similar specimens from Indiana. They provided additional details of this species based on morphological analysis of the type and comparisons with their own fresh collections. *Lactarius cinnabarinus* is characterized by its truffle-like fruiting bodies that have a reddish-orange peridium and a greyish-orange labyrinthoid gleba. When fresh basidiomata are cut, they exude a white latex that does not change color after exposure to air. Sundberg & Trappe (1975) found that *L. cinnabarinus* has subglobose spores 13.0–17.3(–19.0) × (11.0–)12.4–15.4(–17.3) µm (including ornamentation) with reticulated amyloid ornamentation. Two characteristics have caused confusion throughout the history of this species: the size of the spores and the presence of cystidia. In the original description, Singer & Smith (1961) cited ellipsoid spores 14.0–17.0 × 11.0–13.0 µm with a prominent, amyloid reticulum up to 1 µm high, broadly fusoid to ovate acute cystidia (26.0–30.0 × 9.0–13.0 µm) and basidia that are mostly 2–spored (20.0–26.0 × 9.0–12.0 µm). Based on their new collections and re-examination of the type, Sundberg & Trappe (1975) described smaller spores (13.0–16.0 × 12.4–14.8 µm) than those reported by Singer & Smith (1961) and they also clarified that their measurements included the spore ornamentation. They also found that basidia were mostly 2-spored, but noted that some basidia were 1– to 4–spored. Sundberg & Trappe (1975) were not able to view the cystidia reported by Singer & Smith (1961) and they suggested that the cystidia observed in the original description might correspond to unispored basidia at different stages of development.

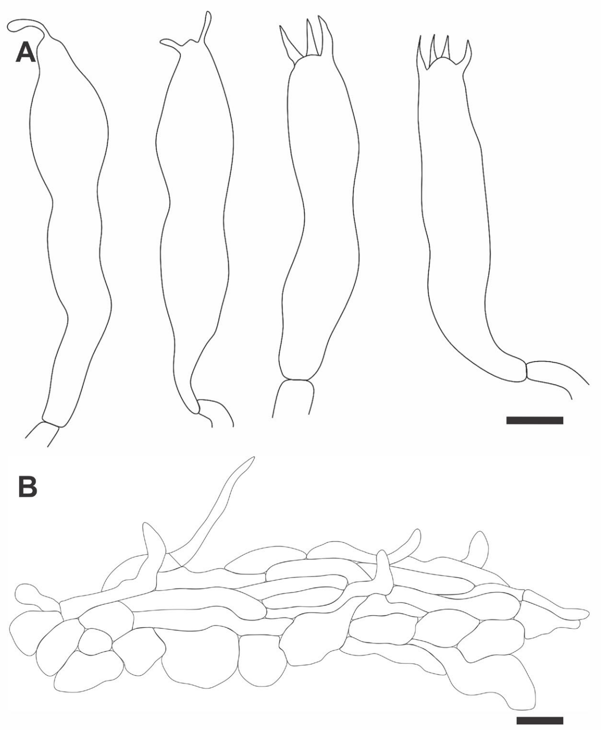

Figure 4. *Lactarius cinnabarinus* line drawings of microscopic structures: A. four types of basidia. B. Detail of the peridiopellis. Scale = 10 µm.

Here, we revisit the identity and distribution of *L. cinnabarinus* based on new molecular data and specimens from across the native range in the southeastern USA, as well as from Argentina and Brazil. Our analysis recovered a well-supported *L. cinnabarinus* clade that included morphologically similar specimens from a wide geographic area in the southeastern USA, including samples from Florida, Georgia, Louisiana, and North Carolina, that was resolved within *Lactarius* subg. *Russularia*. Although we did not sequence additional collections from further north in the USA, specimens and observations cited on iNaturalist (inaturalist.org) and the Mycoportal (mycoportal.org) suggest that the distribution of *L. cinnabarinus* probably extends even further north to Pennsylvania and west to Texas.

Our phylogenetic analysis also showed that a new specimen from Argentina and the recently named species *Lactarius taedae* from Brazil fall into the well supported *L. cinnabarinus* clade. Silva-Filho et al. (2018) recently proposed *L. taedae* as a new species associated with *Pinus taeda* in Brazilian pine plantations. These authors differentiated *L. taedae* from *Z. cinnabarinus* due to smaller spores (9–12.5 × 9–11.5 µm, excluding ornamentation) in *L. taedae* versus the larger spores (14–17 × 11–13 µm) cited by Singer & Smith (1961) for *Z. cinnabarinus*. However, when accounting for the fact that spore ornamentation is variable and that some authors have measured ornamentation and others have not, the spores of *L. taedae* from Brazil are within the range of spore sizes observed by Sundberg & Trappe (1975) for the type specimen and their collections from Indiana, USA. The spore sizes cited by Silva-Filho et al. (2018) are also within the range of those we observed from specimens across the southeastern USA and Argentina. Despite the slight variations in spore sizes across collections from different geographic locations, the remaining morphological characters are congruent with what we observed in the type specimen, and the molecular phylogenetic data suggest that *L. cinnabarinus* is a single, widespread species that forms a well-supported monophyletic clade (Fig. 1).

All of the new *L. cinnabarinus* specimens were collected at sites where pines were present, whether in pure stands, mixed forests, or plantations. This consistent association with pines across both the native and introduced range, along with an ectomycorrhizal root tip sequence of *L. cinnabarinus* from *Pinus taeda* (from Mississippi, Fig. 1), confirms the symbiotic association between *L. cinnabarinus* and pines. Our observations of *L. cinnabarinus* at two different sites in Argentina and Brazil indicate that this fungus was introduced from North America and may be widespread with pines in disturbed environments and plantations across South America.

In order to recognize a monophyletic *Lactarius*, we recombined *Z. cinnabarinus* into the genus *Lactarius* as *L. cinnabarinus*. Publicly available sequence data also indicate that two additional sequestrate species are appropriate to recombine within the genus *Lactarius* (*Z. claridgei* and *Z. corkii*) (Fig. 1). Here, we transferred the type species *of Zelleromyces*, *Z. cinnabarinus*, into the genus *Lactarius* so additional taxonomic work will be needed to determine the appropriate placement of the remaining *Zelleromyces* species. As discussed above, many former *Zelleromyces* species have already been recombined into the genus *Lactarius* (e.g., *L. josserandii* and others – see introduction). Although it is likely that most of the 15 remaining species currently treated in the genus *Zelleromyces* belong to the genus *Lactarius*, as we infer for *Z. claridgei* and *Z. corkii*, it is clear from previous studies that some taxa that morphologically belong in *Zelleromyces* are in fact members of *Russula* or *Lactifluus* rather than *Lactarius* (Lebel 2001; Vidal et al. 2019; Lebel et al. 2021). Evidence suggests that sequestrate basidiomes evolved repeatedly in all three of these genera (Calonge & Martín 2000; Nuytinck et al. 2003; Buyck et al. 2008; Looney et al. 2018; Lebel et al. 2021), a pattern which is common across many groups of fleshy ectomycorrhizal *Basidiomycota* (Kuhar et al. 2023). Accordingly, we refrain from making further taxonomic changes for the remaining *Zelleromyces* species now and we affirm that it will be important to study types and obtain additional DNA sequences of sequestrate *Russulaceae* to resolve their final phylogenetic and taxonomic placement.

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